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Poly(vinyl alcohol)-coated silver nanoparticles: Activation of neutrophils and nanotoxicology effects in human hepatocarcinoma and mononuclear cells

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ABSTRACT

Silver nanoparticles (AgNps) have been described as important for their excellent biocompatibility, biomedical applications. Nevertheless, AgNps can interact with the immune system which is essential to analyze human exposure to assess their potential risk to health and environment. In general, the primary site for accumulation of nanoparticles has been demonstrated to be the liver. Furthermore, the direct activation of neutrophils or oxidative burst by a given nanoparticle is poorly documented. In this paper, we investigated the cell uptake, apoptosis, necrosis, DNA damage in human hepatocarcinoma cells (HepG2), primary normal human peripheral blood mononuclear cells (PBMC) and the direct activation of primary isolated neutrophils through the oxidative burst on exposure to AgNps coated with Polyvinyl-alcohol (PVA). All cell types were incubated in the presence of 1.0 and 50.0 μM of AgNps-PVA for 24 h. A significant cyto- and genotoxic-response and the activation of human neutrophils were induced by AgNps-PVA ($p < 0.05$). Our results revealed that AgNps can interact with the normal isolated neutrophils, PBMC and HepG2 cells *in vitro*, which opens the way for further studies on the toxicological effects of AgNps in the human immune system response and cancer cells.

1. Introduction

There has been a growing number of scientific papers in the last decade with the objective of understanding the interactions between different types of nanoparticles (Nps) and cells (Lewinski et al., 2008). Silver nanoparticles (AgNps), for example, show potential for many biomedical applications, owing to their important bactericidal properties (Asharani et al., 2009). A wide range of AgNps are reported to have potential uses in medical products, such as contraceptive devices, wound dressings, and linings catheter, increasing human exposure and the risk related to their toxicity (Asharani et al., 2009). Some products containing AgNps are applied to broken skin gaining access to systemic circulation, coming in contact with blood cells and reaching other organs including liver or brain (Tang et al., 2009). Despite their widespread use, the impact of

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these Nps remains undefined, and as a result, nanotoxicology is an area of research of great relevance.

Since the exposure route to AgNps may occur through the dermatologic, inhalation or ingestion pathway, as well as with the use of medical devices (Oberdörster et al., 2005), it is relevant to investigate the toxicity of AgNps in relevant human tissues. In general, as the primary site for accumulation of Nps has been demonstrated to be the liver (Sonavane et al., 2008), allowing greater uptake and selective accumulation for therapy, we also decided to investigate the toxicology effects of AgNps in human hepatocarcinoma cells (HepG2). Besides, there are very few studies on the safety and toxicology of AgNps exposure, especially on primary normal human immune system blood cells, such as peripheral blood mononuclear cells (monocytes and lymphocytes – PBMC) and polymorphonuclear neutrophils cells (PMN).

Neutrophils or polymorphonuclear neutrophils (PMNs) are the most abundant leukocytes in circulation and the major phagocytic cells in the blood stream exerting microbicidal role in the host defense against foreign microorganisms. Its antimicrobial function occurs by releasing proteolytic enzymes and also by promoting the oxidative burst characterized by reactive oxygen species (ROS) generation (Clark, 1999). On the other hand, upon activation, the neutrophils also play an important role in inflammatory conditions involved in deleterious effects in chronic inflammation diseases due to the excessive ROS production (Paino et al., 2009). Furthermore, the direct activation of neutrophils by a nanoparticle or its capacity to activate the oxidative burst is still poorly understood, or it has not been reported in the literature in human neutrophils by AgNps-PVA. Since studies investigating the toxic effects of Nps on immune system blood cells are scarce, the understanding of the interactions between nanomaterials and immune system cells is still inconclusive for health application or safety recommendations.

The production of ROS has been reported to contribute to the toxicity of nanoparticles (Marano et al., 2011). ROS can oxidize cell membranes, destabilize mitochondria and activate a wide variety of cellular events, such as inflammation (Paino et al., 2005), apoptosis (Mammucari and Rizzuto, 2010), leading to DNA damage (Asharani et al., 2009; Kawata et al., 2009) and necrosis.

Therefore, the purpose of this study was to investigate the immunological impact of AgNps-PVA in the induction of innate immunity with a focus on oxidative burst from neutrophils. The ROS generation by human normal isolated neutrophils from healthy blood human volunteers was investigate to verify the neutrophils activation by AgNp-PVA and its capacity to activate the oxidative burst, an immune innate defense response. Our study also included a genotoxicity evaluation, *via* the alkaline version of the comet assay, the apoptotic and necrotic effects in primary normal PBMC and HepG2 induced by engineered AgNp-PVA.

2. Materials and methods

2.1. Chemicals

Chemicals, Dulbecco phosphate buffered saline, and Histopaque® 1077, 1119 were purchased from Sigma–Aldrich (USA). Penicillin/streptomycin, fetal bovine serum, and cell culture medium were purchased from Cultilab® and Vitrocell® (Campinas, SP, Brazil). Materials for human blood draw were purchased from BD® (São Paulo, SP, Brazil). All experiments were carried out in a clean and sterile atmosphere to eliminate the probability of endotoxin contamination (Vallhov et al., 2006) in the nanoparticles. All the reagents used for buffer preparation were of analytical grade.

Table 1 – DLS analysis of particle size and zeta potential of PVA-covered AgNp before and after dilution in cell culture medium supplemented with 10% FBS.

AgNp-PVA	As prepared	In cell culture medium with 10% FBS
Zeta potential (mV)	−16.40 ± 8.26	−16.16 ± 1.98
Size (nm)	4.04 ± 0.86	11.70 ± 4.16

Data are reported as means ± standard deviation (SD) of triplicate experiments.

2.2. Synthesis and characterization of AgNps-PVA

$AgNO_3$ (1 mmol L^{-1}) was dissolved in 30 mL of ultrapure water (MilliQ-Plus®) in which 30 mL of PVA 1.0 g L^{-1} was added. AgNps capped with PVA were synthesized by reduction of $AgNO_3$ solution in NaBH4 (0.1 M) under constant stirring for 2 h. The Nps solution was washed by centrifugation at 15,000 rpm for 1 h to remove the excess of reducing agent. The fresh AgNps-PVA were sonicated for 30 min before use and then diluted in cell culture medium.

The average size and zeta potential of AgNps-PVA were determined by dynamic light scattering (DLS, Malvern instruments®, UK) (Table 1). Transmission Electron Microscopy (TEM) images of the AgNPs-PVA were acquired using a FEI Tecnai G2 F20 (TEM/HRTEM) microscope operating at 200 kV (Fig. 1).

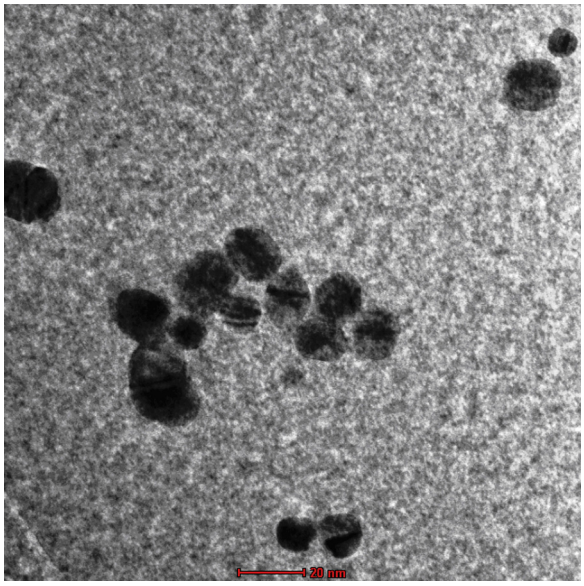

Fig. 1 – TEM images revealing the size and shape of the AgNp-PVA nanoparticles.